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Title: METHOD AND APPARATUS FOR MULTIPLE STAGES OF MASS SPECTROMETRY

FIELD OF THE INVENTION

This invention relates to multiple stage mass spectrometers which have two mass analyzers, and this invention is more particularly concerned with both a method of and an apparatus for providing multiple stages of mass spectrometry (MS^n) capabilities in such spectrometers.

BACKGROUND OF THE INVENTION

Tandem mass spectrometry is widely used for trace analysis and for the determination of the structures of ions. In tandem mass spectrometry a first mass analyzer selects ions of one particular mass to charge ratio (or range of mass to charge ratios) from ions supplied by an ion source, the ions are fragmented and a second mass analyzer records the mass spectrum of the fragment ions. In a triple quadrupole mass spectrometer system, this effects MS/MS. For example, ions produced in an atmospheric pressure source, pass through a region of dry nitrogen and then pass through a small orifice, into a region at a pressure of about 5 torr. Ions then pass through a quadrupole ion guide, operated at a pressure of about $7x10^{-3}$ torr into a first quadrupole mass filter, operated at a pressure of about $2x10^{-5}$ torr. Precursor ions mass selected in the first quadrupole are injected into a collision cell filled with gas, such as argon, to a pressure of 10^{-4} to 10^{-2} torr. The collision cell contains a second quadrupole ion guide, to confine ions to the axis. Ions gain internal energy through collisions with the gas and then fragment. The fragment ions and any undissociated precursor ions then pass into a second mass analyzer, and then to a detector, where the mass spectrum is recorded.

Triple quadrupole systems are widely used for tandem mass spectrometry. One limitation is that recording a fragment mass spectrum can be time consuming because the second mass analyzer must step through many masses to record a complete spectrum. To overcome these

selected in a first mass analyzer and dissociated to produce fragment ions. A fragment ion of a particular mass to charge ratio is then isolated and dissociated again to produce fragments of the fragment. The mass spectrum of these is then recorded. Multiple stages of MS are useful when insufficient dissociation can be produced in a first stage of MS/MS or to elucidate dissociation pathways of complex ions. The latter for example is especially useful to sequence peptides and other biomolecules by mass spectrometry.

The triple quadrupole system and QqTOF system described above provide only one stage of MS/MS and do not allow MS^n. In particular such systems do not provide for trapping of ions.

In one known proposal, in PCT application WO 98/06481 from Analytica of Brantford, there is a described system including ion trapping. Ions from a source are injected into a multipole ion guide, and ions of one m/z or range of m/z are then isolated in the ion guide by applying resonant excitation or AC/DC voltages to the ion guide and trapping voltages at either end. The ion is then fragmented in the same ion guide, which can be operated as a linear ion trap (LIT). No mass analyzer is placed before the ion guide. This is a distinct disadvantage, since a multipole ion guide used both for ion isolation and mass analysis has a relatively low resolution. For example, the present inventors have found that using a LIT as described by Analytica the resolution in isolating an ion is *ca.* 100. With a separate quadrupole mass filter or other mass analyzer before the ion trap the resolution can be many thousand. The relatively low resolution for ions introduced into the multipole ion trap may derive from at least two sources: (1) the pressure is relatively high (10^{-3}-10^{-1} torr as described in the PCT application); and (2) in the system described in the PCT application the gas is either nitrogen or air that flows in from the ion source. This has a greater damping effect on ion motion in the LIT than lighter gases such as helium, and gives relatively poor resolution for resonant excitation of ions. Such a system does not readily enable the pressure and type of gas in the LIT to be adjusted to provide optimum conditions for MS^n.

Additionally, there have been some recent examples of proposals using resonant excitation in RF-only quadrupoles for CID with fragment mass analysis by TOFMS. Dodonov et al (Rap. Comm. Mass Spectrometry 11, 1649-1656 (1997)) introduced a molecular ion reactor (MIR) consisting of a segmented RF-only quadrupole with a longitudinal electrical field which is operated at a high pressure. Depending on the mode of operation, CID was accomplished through either increasing the RF or DC voltages along the segments. However, no trapping of ions was demonstrated.

Loboda et al (proceedings of the 46th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando, Florida, May 31-June 4, 1998, MOD. 11: 55) modified the RF drive of the collision cell in a Q-TOFMS to apply quadrupolar excitation to ions flowing through the cell, inducing fragmentation. No trapping of ions was demonstrated. It was suggested that a 2D trap might be formed to isolate precursor ions, but it was not stated if this was to be done before or after a stage of mass analysis.

J.D. Watson et al, in an article entitled *"A Technique for Mass Selective Ion Rejection in a Quadrupole Reaction Chamber"* (International Journal of Mass Spectrometry and Ion Processes, 93, 225-235, 1989) described trapping and resonant ejection of an ion from the quadrupole collision cell of a triple quadrupole mass spectrometer. The intent was to study reaction kinetics of trapped ions. While there is no specific teaching of resonant excitation of trapped ions without ejection, there is speculation that this might be possible.

In mass spectrometry, the linear ion trap has remained relatively unexplored. U.S. patents 4,755,670 and 5,420,425, both assigned to the Finnigan Corporation, relate to a Fourier transform quadrupole and new ion trap geometries respectively, and they both mention a LIT. U.S. patent 5,179,278 (D.J. Douglas) suggests using a LIT as an "ion bottle" to improve the duty cycle of a 3D ITMS.

SUMMARY OF THE INVENTION

In accordance with a first aspect of the present invention, there is provided a method of analyzing a stream of ions, the method comprising:

(1) subjecting a stream of ions to a first mass analysis step, to select ions having a mass-to-charge ratio in a first desired range;

(2) passing ions in the selected range into a radio frequency linear ion trap containing gas;

(3) trapping the selected ions in the linear ion trap and exciting the ions to cause collisions with the ambient gas and fragmentation;

(4) passing the ions out of the linear ion trap and subjecting the ions to a further mass analysis step to determine the mass spectrum of the ions.

Passing the ions, in step (2) into the radio frequency ion trap can be done either: with a relatively low energy, so no fragmentation occurs in the LIT until additional excitation is applied; or with a relatively high energy in the axial direction, so that fragmentation occurs simply due to the high energy of the ions entering the LIT and colliding with the gas.

Thus a variant of the basic method of the present invention comprises passing the ions into the linear ion trap with sufficient energy to promote collision induced dissociation, said energy providing the excitation of (3), whereby step (3) comprises applying a signal to the linear ion trap to trap ions, before subjecting the ions to the further mass analysis of step (4).

In either case, once the ions have entered the LIT, have been excited, by either technique, to cause fragmentation, one then has fragment ions, with any remaining precursor ions trapped in the LIT. These ions can then be discharged for further mass analysis, or subject to multiple steps of mass selection and excitation to cause fragmentation, before being discharged for the final mass analysis step.

Thus, the method advantageously includes, between steps (3) and (4), subjecting the fragmented ions to a secondary excitation, different from the first excitation, to cause excitation and fragmentation of

selected fragment ions (MS^3). This can be repeated to achieve further steps of MS^n (n greater than 3). Further, prior to the additional step of secondary excitation, applying a signal to the linear ion trap, to select ions having a mass-to-charge ratio in a second desired range, wherein the secondary excitation step comprises exciting ions in the second desired range.

Thus, the method can include, while trapping the ions in the linear ion trap, effecting multiple cycles of:

(1) selecting ions having a mass-to-charge ratio in a desired range; and

(2) exciting the selected ions to cause fragmentation.

The ions can be excited in the linear ion trap by providing an additional signal to the linear ion trap.

The further mass analysis step of step (4) can be carried out either in a quadrupole mass analyzer, or in a time of flight mass analyzer. For a time of flight mass analyzer, this can be arranged with its axis perpendicular to the axis of the linear ion trap.

Preferably, the first mass analysis step is carried out in a quadrupole mass analyzer which is coaxial with the linear ion trap.

More preferably, the method includes, prior to exciting the ions in step (3), subjecting the trapped ions to a signal comprising a plurality of excitation signals uniformly spaced in the frequency domain and having a notch, wherein the notch covers a desired frequency band and there are no excitation signals in the frequency band of the notch, and wherein the excitation signals have sufficient magnitude to excite and eject ions except for ions having an excitation frequency falling within the frequency band of the notch. For the case where the frequency of the trapping RF signal is 1.0 MHz, this can be achieved by applying a combination of signals having sine waves with frequencies in the range 10 to 500 kHz and spaced at 500 Hz intervals, and the frequency band of the notch then has a width of typically 1-10 kHz and is centered on the resonant frequency of an ion of interest. More generally, where the trapping RF frequency is f, then the auxiliary frequencies should be up to f/2.

against frequency.

Figures 7c and 7d are graphs showing variation of intensity against frequency for different pressures in the chamber of Figure 2b;

Figure 7e is a graph showing variation of resolution with pressure for different excitation voltages for the apparatus of Figure 2b;

Figure 8 is an isometric 3-dimensional view showing variation of the intensity of reserpine precursor ion with the auxiliary voltage;

Figures 9a and 9b are graphs showing similar plots to Figure 8 with the auxiliary voltage at the resonant frequency and 2-5 kHz below resonant frequency;

Figure 10 shows a variation of precursor and fragment intensity with excitation period; and

Figure 11 is a series of graphs demonstrating MS^3 in the apparatus of Figure 2b.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Referring first to Figure 1, a mass spectrometer is indicated generally by the reference 10. Ions are generated by an ion source 12, which is a pneumatically assisted electrospray, and pass through a dry nitrogen "curtain gas", indicated at 14. The ions then pass through an orifice in plate 16, and then through a further orifice in a skimmer 18, into a first quadrupole rod set Q0.

The rod set Q0 is located in a first chamber 22 which is connected to a turbo molecular pump, with the connection indicated at 24. Although not shown, in known manner, the turbo molecular pump 24 is backed up by a rotary vane pump, which can also be connected to the region between the orifice plate 16 and the skimmer plate 18. Alternatively the region between the orifice and skimmer plates 16, 18 can be evacuated by a separate rotary vane pump.

The turbo molecular pump 24 maintains a pressure of 7×10^{-3} torr in the chamber 22, while a pressure of 2 torr is maintained between

the orifice and skimmer plates 16, 18. The rod set Q0 has just an RF voltage applied to it, so that it operates as an ion guide.

Ions then pass through into a main chamber 26 of the mass spectrometer. Within the main chamber 26, there are located first, second and third quadrupole rod sets, indicated at Q1, Q2 and Q3. A detector 36 is provided at the exit from the final rod set at Q3.

As indicated at 30, a connection to a suitable turbo molecular pump would be provided, again backed by the same rotary vane pump that backs turbo molecular pump 24. The pump 30 maintains a pressure of 2×10^{-5} torr in the main chamber 26.

The central quadrupole rod set Q2 is enclosed in a chamber or housing 28 and is provided with a connection for a gas (not shown), so that a higher pressure can be maintained typically at around 1-7 millitorr.

Now, in accordance with the present invention, the housing or enclosure 28 with the rod set Q2 forms a linear ion trap. For this purpose, conductive plates with apertures are provided at the ends of the housing 28, which may be either separate from the housing 28 or integral therewith. These comprise an entrance plate 32 and an exit plate 33. The plates 32, 33 are conductive, insulated from another and connected to voltage sources 34.

Downstream from the housing 28 is a third quadrupole rod set, Q3, configured as a mass analyzer. For operation as a conventional triple quadrupole MS/MS system, the quadrupole rod sets Q0, Q1, Q2 and Q3 would be connected to conventional voltage sources, for supplying DC and RF voltages as required.

In use, ions generated from the ion source 12 pass into the quadrupole ion guide Q0. As noted, this is supplied with just RF voltages, to operate as an ion guide. Ions then pass through Q0 into the first quadrupole rod set Q1. This is supplied with suitable RF and DC voltages to operate as a mass filter, to select ions with a desired m/z ratio.

A mass selected precursor ion from the first rod set Q1 is then injected into the collision cell 28, to produce fragment ions as is

brevity, like components in Figure 2a are given the same reference numeral as in Figure 1, and description of these components is not repeated.

In Figure 2a, the time of flight device 40 is connected to the exit plate 33 of the collision cell 28. In known manner, the time of flight device 40 includes a connection 42 to a pump for maintaining a vacuum at 5×10^{-7} torr. It includes a repeller grid 44 and other grids indicated schematically at 46, for collecting ions entering the TOF 40 and transmitting a pulse of ions. The TOF device 40 here is a reflectron and includes grids 48 for reflecting the ion beam, which is then detected by a detector 49. A linear TOF may also be used, as shown in Figure 2b.

The apparatus in Figure 2a would be operated in an essentially similar manner to that of Figure 1. The principal difference is that the TOF can record 10^4 or more complete mass spectra in one second. Thus for applications where a complete mass spectrum of fragment ions is desired the duty cycle is greatly improved with a TOF mass analyzer 40 and spectra can be acquired more quickly. Alternatively, for a given measurement time, spectra can be acquired on a smaller amount of sample.

While three-dimensional (3-D) traps (IT) have been provided in spectrometers including a TOF final stage, a two-dimensional (2-D) trap has several advantages over the 3-D trap. Firstly, because there is no quadrupolar electric field in the z direction, the ion injection and extraction efficiencies can be nearly 100%. As fewer ions are lost in the processes of filling and emptying the trap the sensitivity of the Linear Ion Trap Time Of Flight Mass Spectrometer (LIT/TOFMS) can be greater than that of the IT/TOFMS (an ESI source, a 3-D ion trap mass spectrometer and a TOFMS).

Because of the increased trapping volume of the LIT, a greater number of ions ($N_{2\text{-}d}$) can be trapped in a LIT than in a 3-D trap ($N_{3\text{-}d}$). The increase in ion capacity is given by

$$\frac{N_{2-d}}{N_{3-d}} = \frac{r_0^2 l}{z_0^3} \qquad (1)$$

Reference will now be made to Figure 2b, which shows an alternative embodiment. This was designed without the initial, mass resolving quadrupole Q1, to provide experimental data on the performance of the LIT. It also includes a linear TOF section, to provide LIT/TOFMS.

The LIT/TOFMS was designed to be flexible with three modes of operation: (i) continuous flow-TOFMS, in which the products of ESI can be analyzed without trapping or fragmentation; (ii) trap-TOFMS, in which the combination of trapping and pulsing ions can be used to enhance instrumental duty cycle; and (iii) MS/MS-TOFMS in which the fragmentation spectra for isolated precursor ions are recorded via TOFMS. Switching between modes is a simple matter of changing the parameters which control timing, trap entrance and exit potentials, and excitation frequencies and amplitudes.

In Figure 2b, the spectrometer is indicated generally at 50. Ions are generated by pneumatically assisted electrospray at 52 and pass through a dry nitrogen curtain gas 54, a 0.25 mm diameter sampling orifice in an orifice plate 56, a 0.75 mm diameter orifice in the skimmer 58, and into a first RF-only quadrupole Q0. The region between the skimmer and the orifice is evacuated by a rotary vane pump as indicated at 62, to a pressure of 2 torr. A second quadrupole rod set is indicated at Q2. For consistency with Figure 2a, the designation Q2 is used, although there is no Q1 in Figure 2b.

The RF-only quadrupoles Q0, Q2 are separated by a 1mm diameter interquad aperture 64 (IQ). The first quadrupole, Q0, is 5 cm long and the second Q2, which acts as the LIT, is 20 cm long. Both quadrupoles have field radii of 4.0 mm and are operated by the same main RF drive, which has a maximum V_{rf} of 5000 V and a drive frequency, $\Omega = 2\pi f$ where f is 1 MHz. There are 12.5 pF capacitors between the RF drive supply and the rods of Q0; thus the RF voltage on Q0 is ca. one half that of Q2. The DC offsets of the quadrupoles are individually set and for all the experiments here these are Q0=10 V and Q2=0 V. The pressure in the LIT can be varied from 1.5 to 7.0 mTorr by adding gas. The region surrounding the LIT

provided by Q2 is connected to a turbomolecular pump, as indicated at 66. The LIT chamber is indicated at 68.

A TOF chamber 70 is coupled orthogonally to the LIT chamber 68 via four lenses, L1-L4. L1 (aperture diameter 0.75 mm) serves as the exit of the LIT chamber 68 and the differential pumping aperture between the LIT and the TOF chambers. The three lenses, L2, L3 and L4, have apertures of 2 mm diameter and are used to focus the ion beam into the source region of a two stage, 1 m long, TOFMS. The TOF chamber 70 is held at a pressure of 1.2×10^{-6} torr or less by a turbomolecular pump. Separate rotary vane pumps are used to pump the region between the orifice and skimmer and to back the turbo pumps.

In the TOF source region, in known manner there are a repeller grid 72, a middle TOF grid 74 and a final TOF grid 76. The ion source was operated near ground potential and the flight tube was floated at a negative high potential, typically 2.0kV. To reduce distorting effects of the floating voltage a shielding grid 78 was placed 4.2 mm behind the middle TOF grid 74. An additional shielding grid 80 was placed around the repeller grid 72 and the middle TOF grid to reduce the effects of stray fields on ions entering the source region. Ions are accelerated in the TOF in a direction orthogonal to that of the quadrupoles. Thus, the system is termed an orthogonal acceleration TOF (oa-TOF).

There is no potential difference between the Q2 offset and the source region, thus the axial ion energy in the TOF source is < 1 eV. The repeller grid 72 of the TOF is pulsed from an offset of 0 V to an amplitude of $\approx$ 200-300 V using a high voltage (HV) pulser (rise time < 18 ns). The amplitude of the HV pulse is adjusted to achieve maximum resolution for the ion acceleration energy. Because the ions enter the source region midway between the repeller grid 72 and grid 74, the acceleration energy is given by one half of the amplitude of the HV pulse minus the negative float potential. The experimental HV pulse amplitudes that gave the best resolution were found to equal those calculated to give space focussing for the set acceleration energies. The HV pulse width is set to be greater than

substantial loss in resolution in the TOFMS. Nitrogen was added to the flight tube to increase the pressure over the range 1.2×10^{-6} torr to 5×10^{-5} torr, corresponding to a decrease in the mean free path for the +13 charge state of cytochrome *c* (collision cross section ~ 1700 Å^2) from ~ 106 cm to ~ 4 cm. The resolution in the TOFMS spectrum degraded from 600 to 30 with a ca. 25 x increase in the number of collisions. At 1.2×10^{-6} torr, the probability of the ion undergoing zero collisions in the flight path is 52% and of one collision 34%. Extrapolation of a graph of resolution vs. λ^{-1} shows that at zero pressure the resolution is improved ca. 6% over that of 1.2×10^{-6} torr.

A schematic of the RF operation for the LIT is shown in Figure 3. The master clock for the LIT/TOFMS is provided by a two channel arbitrary waveform generator 82 (AWG). Each channel of the AWG 82 provides a maximum amplitude (0 to peak) of 12 V. The AWG 82 is connected to an auxiliary drive (Aux. Drive) 84, which in turn is connected by a bipolar transformer 85 to the *y* rods. A main RF drive 86, as shown, is connected directly to the *x* rods, with one connection being through the transformer 85 to the y rods.

As shown in Figure 4 the complete MS/MS cycle takes 20 ms to complete. It involves changing the potentials on the interquad aperture (IQ) 64 and exit aperture L1, control of the auxiliary driver 84 which connects the output of the AWG 82 to the quadrupole rods Q2, and the TOFMS pulsing (TOF).

The first phase of the cycle is ion injection. A synchronization pulse from the AWG 82 triggers a pulse generator (not shown) which controls the potential on IQ 64, which is maintained at a potential (~7 V) indicated at 100 to pass ions for a set injection time (typically 5 ms as shown in Figure 4) and a stopping potential 102 (12 V) for the remaining 15 ms of the scan. In addition this injection time serves as a thermalization period. For the ions that were studied, fragmentation spectra were independent of orifice skimmer potential difference, suggesting that any ion heating in the ion sampling region has equilibrated during the

7b with q_y=0.51, only ions with $\frac{m}{z}$ >345 will have a Mathieu parameter for motion in the y direction, q_y, of <0.9 and thus stable trajectories in the trap. Lower mass fragments will have unstable trajectories and thus, even if formed by the resonance excitation process, cannot be detected in the TOFMS. This decreases the fragmentation efficiency, but this is an inherent feature of CID in any ion trap.

Figure 7c and 7d compare resonant excitation curves, which show precursor and fragment ion intensities for renin substrate as a function of the frequency, ω_a of the auxiliary voltage for ω_a near ω_o (the fundamental resonant frequency of the system) for pressures of 7 mTorr (a) and 1.5 mTorr (b) respectively in the chamber 68. The data of Figure 7c is the same as Figure 7a, and references 120c, 120d, 124c, 124d are used to identify the curves in these Figure 7c, 7d. In each case a broadband waveform constructed to isolate the precursor ion, (the +3 charge state of renin substrate $\frac{m}{z}=587$), was applied for 4 ms prior to the irradiation with the auxiliary voltage. Figure 7c again shows, for a pressure of 7 mTorr the intensity of the precursor ion 120c falling to a minimum, as the intensity of the sum of the fragments 124c reaches a maximum. Corresponding curves 120d, 124d are shown in Figure 7d, for the precursor ions and the fragment ions for operation at 1.5 mTorr pressure. The achieved resolution at 7 mTorr was ~70 and at 1.5 mTorr was approximately ~230. The major difference in the excitation parameters for the two pressures is the amplitude of the auxiliary voltage. At 7 mTorr a 0-peak voltage of 1500 mV was required to achieve fragmentation and ejection while at 1.5 mTorr the same phenomena were observed with 300 mV.

Figure 7e demonstrates the achieved resolutions for different excitation voltages over a range of pressures. Resolution remains essentially constant as a function of pressure at each amplitude. Clearly the use of a lower auxiliary voltage amplitude is the dominant factor in the observed improved resolution at the lower pressure.

ion ejection and ion fragmentation. For ejection the important parameters are q_y, the mass and charge of the precursor ions, the pressure and mass of the collision gas, and the collision cross section of the precursor ion, as well as the duration and amplitude of the auxiliary voltage. Fragmentation, however, is also dependent upon the structure of the precursor ion, the effectiveness of the transfer of kinetic energy to internal energy through collisions, and the time scale for the unimolecular dissociation of the precursor ion. The details of this competition have been discussed for 3-D ITMS previously. There follows a discussion on tests to demonstrate how operating parameters for the apparatus of Figure 2b affects competition between ejection and fragmentation.

Figure 8 shows the effect on singly charged reserpine ions of increasing the amplitude of the auxiliary voltage, as plotted against intensity and channel number. While a threshold voltage is necessary to induce fragmentation, as the amplitude increases ejection dominates and no fragmentation is observed. The precursor ion is indicated at 130, and fragments at 132, 134. Figure 9a shows a similar plot for the same experiment for the +3 charge state of renin substrate, with the precursor indicated at 136 and the sum of the fragments at 138. Figure 9b demonstrates the effect of increasing the amplitude of the excitation voltage at $\omega_a=\omega_o$-2.5 kHz, with the precursor indicated at 140 and the sum of the fragments at 142. The extent of fragmentation increases by 50% and the excitation amplitude for which fragmentation is one half of its maximum value increases by 40% when off resonant excitation is used. Similar increases in the extent of fragmentation and the amplitude for which fragment ions could be detected were observed for all ions and charge states examined.

The fragmentation spectrum recorded with low amplitude resonant excitation (A_a=1.5 V, $\omega_a=\omega_o$) was similar to that recorded in a triple quadrupole mass spectrometer system with 15 eV laboratory injection energy and a 20 cm long collision cell filled with 1 mTorr of Krypton (The triple quadrupole is described in Y-L. Chen, B.A. Collings, and D.J. Douglas,

with other ions being ejected. Finally, the isolated peak is fragmented through the application of a low amplitude sinusoidal oscillation for another 1 ms. The fragments of the isolated fragment at $\frac{m}{z} = 726$ are shown in Figure 11e. The total trapping time for the MS^3 process was 10 ms, giving a cycle time for MS^3 of 22 ms, with 70 TOFMS scans in each MS^3 cycle. As is shown the spectral intensity is lower by a factor of 100 in the MS^3 process.

Nitrogen was used as the collision gas because it flowed into the quadrupole from the curtain gas region. A pressure of 7 mTorr was initially used because this previously was found to give optimum collisional focussing for a single pass through an RF quadrupole of similar length. These choices however, somewhat limited the performance of the LIT. There are experimental tradeoffs when using a heavier neutral as a collision gas. The heavier neutral results in a larger center of mass collision energy for a given ion energy, and thus both a greater energy exchange in collisions and more scattering. While the larger collision energy exchange results in fragmentation occurring on a shorter time scale, the increase in the extent of scattering can degrade excitation resolution. The inelastic collisions between the gas and the precursor ion act as a "frictional force" which dampens the forced oscillation of a harmonic system and the width of the power absorption is related to the dampening of the ion motion. Lowering the pressure and mass of the gas is expected to lower the frictional force, thus narrowing the width of the power absorption and thereby increasing the possible excitation resolution. This applies to both the broadband excitation waveform and the resonant excitation resolution.

CLAIMS:

1. A method of analyzing a stream of ions, the method comprising:

(1) subjecting a stream of ions to a first mass analysis step, to select ions having a mass-to-charge ratio in a first desired range;

(2) passing ions in the selected range into a radio frequency linear ion trap containing a gas;

(3) trapping the selected ions in the linear ion trap and exciting the ions to cause collisions with the gas and fragmentation;

(4) passing the ions out of the linear ion trap and subjecting the ions to a further mass analysis step to determine the mass spectrum of the ions.

2. A method as claimed in Claim 1, which includes, between steps (3) and (4), subjecting the fragmented ions to a secondary excitation, different from the first excitation, to cause excitation and fragmentation of selected fragment ions.

3. A method as claimed in Claim 2, which includes, prior to the additional step of secondary excitation, applying a signal to the linear ion trap, to isolate ions having a mass-to-charge ratio in a second desired range, wherein the secondary excitation step comprises exciting ions in the second desired range.

4. A method as claimed in Claim 3, which includes, while trapping the ions in the linear ion trap, effecting multiple cycles of:

(1) isolating ions having a mass-to-charge ratio in a further desired range; and

(2) exciting the isolated ions in the further desired range to cause fragmentation.

5. A method as claimed in Claim 1, 2, 3, or 4 wherein step (2) comprises passing the ions into the linear ion trap with sufficient energy to promote collision induced dissociation, the said energy providing the excitation of step (3), whereby step (3) comprises applying a signal to the linear ion trap to trap ions before subjecting the ions to the further mass analysis of step 4).

6. A method as claimed in Claim 1, 2, 3 or 4 which comprises exciting the ions in the linear ion trap by providing an additional signal to the linear ion trap.

7. A method as claimed in Claim 1, wherein the further mass analysis step of step (4) is carried out in a quadrupole mass analyzer.

8. A method as claimed in Claim 1, wherein the further mass analysis step of step (4) is carried out in a time of flight mass analyzer.

9. A method as claimed in Claim 8 wherein the further mass analysis step is carried out in a time of flight mass analyzer arranged with its axis perpendicular to the axis of the linear ion trap.

10. A method as claimed in Claim 1 wherein each mass analysis step is carried out in one of: a linear quadrupole; a linear time of flight analyzer; a reflectron time of flight analyzer; a single magnetic sector analyzer; a double focusing two sector mass analyzer having an electric sector and a magnetic sector; a Paul trap; a Wien filter; a Mattauch-Herzog spectrograph; ion cyclotron mass spectrometer; and a Thomson parabolic mass spectrometer.

11. A method as claimed in Claim 7, 8, 9 or 10, wherein the first mass analysis step is carried out in a quadrupole mass analyzer which is

coaxial with the linear ion trap.

12. A method as claimed in Claim 1, which includes, prior to exciting the ions in step (3), subjecting the trapped ions to a signal comprising a plurality of excitation signals uniformly spaced in the frequency domain and having a notch, wherein the notch covers a desired frequency band and there are no excitation signals in the frequency band of the notch, and wherein the excitation signals have sufficient magnitude to excite and eject ions except for ions having an excitation frequency within the frequency band of the notch.

13. A method as claimed in Claim 12, which comprises applying a combination of signals comprising sine waves and with frequencies up to f/2, where f is the frequency of the trapping RF.

14. A method as claimed in Claim 12, which comprises applying a combination of signals having sine waves with frequencies in the range 10 to 500 kHz and spaced at 500 Hz intervals, and the frequency band of the notch has a width of 1-10 kHz and is centered on the resonant frequency of an ion of interest.

15. A method as claimed in Claim 12, which includes, after selection of a desired ion, exciting the desired ion with a signal comprising a sine wave at or near the resonant frequency of the ion.

16. A method as claimed in Claim 8, which includes providing an exit lens between the linear ion trap and the time of flight device, and lowering the voltage on the exit lens to permit ions to pass into the time of flight device, the method further comprising providing a signal to a repeller grid of the time of flight device, to cause the time of flight device to scan at a desired rate.

17. A method as claimed in Claim 16, which comprises passing ions, in step (2), into the linear ion trap for a period of substantially 5ms subjecting the ions in the linear ion trap to an excitation signal to excite and eject undesired ions for a period of substantially 4ms, exciting the desired ions for a period of substantially 4ms and passing the ions out of the linear ion trap and scanning the time of flight device for substantially 7ms.

18. An apparatus, for effecting mass analysis and fragmentation of an ion stream, the apparatus comprising:

an input for an ion stream;

a first mass analyzer;

a radio frequency linear ion trap; and

a final mass analyzer.

19. An apparatus as claimed in Claim 18, wherein the first mass analyzer comprises a quadrupole mass analyzer.

20. An apparatus as claimed in Claim 19, wherein the final mass analyzer comprises a quadrupole mass analyzer, and the first mass analyzer, the linear ion trap and the final mass analyzer are axially aligned with one another.

21. An apparatus as claimed in Claim 20, wherein the linear ion trap includes a multipole rod set.

22. An apparatus as claimed in Claim 21, wherein the linear ion trap comprises a quadrupole rod set and wherein the rods of the mass analyzers and of the linear ion trap have substantially similar radii and substantially similar spacings.

23. An apparatus as claimed in Claim 19, wherein the final mass analyzer comprises a time of flight device.

24. An apparatus as claimed in Claim 18, wherein each of the first analyzer and the final analyzer comprise one of: a linear quadrupole; a linear time of flight analyzer; a reflectron time of flight analyzer; a single magnetic sector analyzer; a double focusing two sector mass analyzer having an electric sector and a magnetic sector; a Paul trap; a Wien filter; a Mattauch-Herzog spectrograph; an ion cyclotron mass spectrometer; and a Thomson parabolic mass spectrometer.

25. An apparatus as claimed in Claim 24, wherein the linear ion trap includes a multipole rod set.

26. An apparatus as claimed in Claim 18, wherein the linear ion trap has a pair of opposed x rods and a pair of opposed y rods, wherein a main RF drive is connected to the x and y rods of the linear ion trap, and wherein an auxiliary drive is connected to at least one pair of rods of the linear ion trap.

27. An apparatus as claimed in Claim 26, wherein the auxiliary drive is connected to the y rods of the linear ion trap through a transformer, and wherein the main RF drive is connected directly to the x rods of the linear ion trap and, through a coil of the transformer to the y rods.

28. An apparatus as claimed in Claim 26, which includes an arbitrary waveform generator connected to the auxiliary drive, for applying a selected waveform to the linear ion trap to excite ions therein.

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
571-596		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA99/01142	30/11/1999	02/12/1998
International Patent Classification (IPC) or national classification and IPC H01J49/42		
Applicant UNIVERSITY OF BRITISH COLUMBIA et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of twenty sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
30/05/2000	06.04.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Korb, W Telephone No. +49 89 2399 2284

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA99/01142

I. Basis f the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

2,7,8,11,13,14,17, 19-23,25,27	as originally filed			
1,3,4,4a,5,6,9,10, 12,15,16,18,24,26, 28	as received on	28/02/2001	with letter of	28/02/2001

Claims, No.:

1-27	as received on	28/02/2001	with letter of	28/02/2001

Drawings, sheets:

1/14-14/14	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1 - 27
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1 - 27
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1 - 27
	No:	Claims	

2. Citations and explanations
   see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate she t

R Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. There is no doubt in regard of the possibility of an industrial applicability of the subject-matter claimed in claims 1 - 27.

   Furthermore the subject-matter of independent claims 1 and 17 is considered to be new and to involve an inventive step with respect to the available documents cited in the International Search Report and representing a state of the art according to Rule 64(1) PCT.

   The dependent claims 2 - 27 refer to claim 1 or claim 17 directly or indirectly and meet the requirements for such claims with regard to novelty and inventive step.

2. The present application is related to a method of analysing a stream of ions and the corresponding apparatus for effecting mass analysis and fragmentation of an ion stream which apply a particular sequence of steps requiring particular apparatus features.

   The problem to be solved is to overcome limitations of prior art systems.

   The subject-matter claimed is distinguished over the prior art WO-99/30350 by steps 1 and 4 of the method and the related apparatus features. In contrast to the teaching of WO-99/30350 the subject-matter claimed in claim 17 of the present application includes a mass analyser before the radio frequency linear ion trap while WO-99/30350 simply discloses an RF-only ion guide before the radio frequency linear ion trap.

   With regard to WO-97/07530 it has to be noted that this document does not disclose or teach multiple mass spectrometry within the collision cell or Q_2 which is the main concept of the present application. Furthermore the use of ion trapping is not disclosed. In addition, the excitation signals used in the present application to facilitate multiple mass spectrometry, differ from the axial field disclosed in WO-97/07530.

R It m VII

Certain defects in the international application

An independent claim should be properly cast in the two-part form in accordance with Rule 6.3(b) PCT, with those features known in combination from the prior art (document D1) being placed in the preamble (Rule 6.3(b)(i) PCT) and with the remaining features being included in the characterising part (Rule 6.3(b)(ii) PCT).

Re Item VI

Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO-A-99/30350	17.06.99	03.12.98	05.12.97

The disclosure of this document is relevant to the subject-matter of claims 1 and 17.

Title: METHOD AND APPARATUS FOR MULTIPLE STAGES OF MASS SPECTROMETRY

FIELD OF THE INVENTION

This invention relates to multiple stage mass spectrometers which have two mass analyzers, and this invention is more particularly concerned with both a method of and an apparatus for providing multiple stages of mass spectrometry (MS^n) capabilities in such spectrometers.

BACKGROUND OF THE INVENTION

Tandem mass spectrometry is widely used for trace analysis and for the determination of the structures of ions. In tandem mass spectrometry a first mass analyzer selects ions of one particular mass to charge ratio (or range of mass to charge ratios) from ions supplied by an ion source, the ions are fragmented and a second mass analyzer records the mass spectrum of the fragment ions. In a triple quadrupole mass spectrometer system, this effects MS/MS. For example, ions produced in an atmospheric pressure source, pass through a region of dry nitrogen and then pass through a small orifice, into a region at a pressure of about 5 torr (0.7 kPa). Ions then pass through a quadrupole ion guide, operated at a pressure of about $7x10^{-3}$ torr ($9.1x10^{-4}$ kPa) into a first quadrupole mass filter, operated at a pressure of about $2x10^{-5}$ torr ($2.6x10^{-6}$ kPa). Precursor ions mass selected in the first quadrupole are injected into a collision cell filled with gas, such as argon, to a pressure of 10^{-4} to 10^{-2} torr ($1.3x10^{-5}$ to $1.3x10^{-3}$ kPa). The collision cell contains a second quadrupole ion guide, to confine ions to the axis. Ions gain internal energy through collisions with the gas and then fragment. The fragment ions and any undissociated precursor ions then pass into a second mass analyzer, and then to a detector, where the mass spectrum is recorded.

Triple quadrupole systems are widely used for tandem mass spectrometry. One limitation is that recording a fragment mass spectrum can be time consuming because the second mass analyzer must step through many masses to record a complete spectrum. To overcome these

selected in a first mass analyzer and dissociated to produce fragment ions. A fragment ion of a particular mass to charge ratio is then isolated and dissociated again to produce fragments of the fragment. The mass spectrum of these is then recorded. Multiple stages of MS are useful when insufficient dissociation can be produced in a first stage of MS/MS or to elucidate dissociation pathways of complex ions. The latter for example is especially useful to sequence peptides and other biomolecules by mass spectrometry.

The triple quadrupole system and QqTOF system described above provide only one stage of MS/MS and do not allow MS^n. In particular such systems do not provide for trapping of ions.

In one known proposal, in PCT application WO 98/06481 from Analytica of Brantford, there is a described system including ion trapping. Ions from a source are injected into a multipole ion guide, and ions of one m/z or range of m/z are then isolated in the ion guide by applying resonant excitation or AC/DC voltages to the ion guide and trapping voltages at either end. The ion is then fragmented in the same ion guide, which can be operated as a linear ion trap (LIT). No mass analyzer is placed before the ion guide. This is a distinct disadvantage, since a multipole ion guide used both for ion isolation and mass analysis has a relatively low resolution. For example, the present inventors have found that using a LIT as described by Analytica the resolution in isolating an ion is *ca.* 100. With a separate quadrupole mass filter or other mass analyzer before the ion trap the resolution can be many thousand. The relatively low resolution for ions introduced into the multipole ion trap may derive from at least two sources: (1) the pressure is relatively high (10^{-3}-10^{-1} torr (1.3×10^{-4} to 1.3×10^{-2} kPa) as described in the PCT application); and (2) in the system described in the PCT application the gas is either nitrogen or air that flows in from the ion source. This has a greater damping effect on ion motion in the LIT than lighter gases such as helium, and gives relatively poor resolution for resonant excitation of ions. Such a system does not readily enable the pressure and type of gas in the LIT to be adjusted to provide optimum conditions for MS^n.

Additionally, there have been some recent examples of proposals using resonant excitation in RF-only quadrupoles for CID with fragment mass analysis by TOFMS. Dodonov et al (Rap. Comm. Mass Spectrometry 11, 1649-1656 (1997)) introduced a molecular ion reactor (MIR) consisting of a segmented RF-only quadrupole with a longitudinal electrical field which is operated at a high pressure. Depending on the mode of operation, CID was accomplished through either increasing the RF or DC voltages along the segments. However, no trapping of ions was demonstrated.

Loboda et al (proceedings of the 46th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando, Florida, May 31-June 4, 1998, MOD. 11: 55) modified the RF drive of the collision cell in a Q-TOFMS to apply quadrupolar excitation to ions flowing through the cell, inducing fragmentation. No trapping of ions was demonstrated. It was suggested that a 2D trap might be formed to isolate precursor ions, but it was not stated if this was to be done before or after a stage of mass analysis.

B.A. Thomson et al, in PCT application PCT/CA96/00541, describes a method and apparatus for speeding up the passage of ions through various stages of a mass spectrometer, such as the ion guide and the collision cell. The increase in ion speed is achieved via an axial DC field which can be created through various multipole rod configurations. The axial DC field also aids in the dissociation of ions in collision cells by oscillating the ions axially about their equilibrium positions. However, Thomson states that there is no need to operate at the resonant frequency of the ions or even at a harmonic of the resonant frequency of the ions.

J.D. Watson et al, in an article entitled *"A Technique for Mass Selective Ion Rejection in a Quadrupole Reaction Chamber"* (International Journal of Mass Spectrometry and Ion Processes, 93, 225-235, 1989) described trapping and resonant ejection of an ion from the quadrupole collision cell of a triple quadrupole mass spectrometer. The intent was to study reaction kinetics of trapped ions. While there is no specific teaching of resonant excitation of trapped ions without ejection, there is speculation that this might be possible.

In mass spectrometry, the linear ion trap has remained relatively unexplored. U.S. patents 4,755,670 and 5,420,425, both assigned to the Finnigan Corporation, relate to a Fourier transform quadrupole and new ion trap

geometries respectively, and th y both mention a LIT. U.S. patent 5,179,278 (D.J. Douglas) suggests using a LIT as an "ion bottle" to improve the duty cycle of a 3D ITMS.

SUMMARY OF THE INVENTION

In accordance with a first aspect of the present invention, there is provided a method of analyzing a stream of ions, the method comprising:

(1) subjecting a stream of ions to a first mass analysis step, to select ions having a mass-to-charge ratio in a first desired range;

(2) passing ions in the selected range into a radio frequency linear ion trap containing gas;

(3) trapping the selected ions in the linear ion trap and exciting the ions to cause collisions with the ambient gas and fragmentation;

(4) subjecting the fragmented ions to a secondary excitation, different from the first excitation, to cause excitation and fragmentation of selected fragment ions; and

(5) passing the ions out of the linear ion trap and subjecting the ions to a further mass analysis step to determine the mass spectrum of the ions.

Passing the ions, in step (2) into the radio frequency ion trap can be done either: with a relatively low energy, so no fragmentation occurs in the LIT until additional excitation is applied; or with a relatively high energy in the axial direction, so that fragmentation occurs simply due to the high energy of the ions entering the LIT and colliding with the gas.

Thus a variant of the basic method of the present invention comprises passing the ions into the linear ion trap with sufficient energy to promote collision induced dissociation, said energy providing the excitation of (3), whereby step (3) comprises applying a signal to the linear ion trap to trap ions, before subjecting the ions to the further mass analysis of step (5).

In either case, once the ions have entered the LIT, have been excited, by either technique, to cause fragmentation, one then has fragmentions, with any remaining precursor ions trapped in the LIT. These ions can then be discharged for further mass analysis, or subject to multiple steps of mass selection and excitation to cause fragmentation, before being discharged for the final mass analysis step.

Thus, the method advantageously includes, in step (4), subjecting the fragmented ions to a secondary excitation, different from the first excitation, to cause excitation and fragmentation of selected fragment ions

(MS^3). This can be repeated to achieve further steps of MS^n (n greater than 3). Further, prior to the additional step of secondary excitation, applying a signal to the linear ion trap, to select ions having a mass-to-charge ratio in a second desired range, wherein the secondary excitation step comprises exciting ions in the second desired range.

Thus, the method can include, while trapping the ions in the linear ion trap, effecting multiple cycles of:

(1) selecting ions having a mass-to-charge ratio in a desired range; and

(2) exciting the selected ions to cause fragmentation.

The ions can be excited in the linear ion trap by providing an additional signal to the linear ion trap.

The further mass analysis step of step (5) can be carried out either in a quadrupole mass analyzer, or in a time of flight mass analyzer. For a time of flight mass analyzer, this can be arranged with its axis perpendicular to the axis of the linear ion trap.

Preferably, the first mass analysis step is carried out in a quadrupole mass analyzer which is coaxial with the linear ion trap.

More preferably, the method includes, prior to exciting the ions in step (3), subjecting the trapped ions to a signal comprising a plurality of excitation signals uniformly spaced in the frequency domain and having a notch, wherein the notch covers a desired frequency band and there are no excitation signals in the frequency band of the notch, and wherein the excitation signals have sufficient magnitude to excite and eject ions except for ions having an excitation frequency falling within the frequency band of the notch. For the case where the frequency of the trapping RF signal is 1.0 MHz, this can be achieved by applying a combination of signals having sine waves with frequencies in the range 10 to 500 kHz and spaced at 500 Hz intervals, and the frequency band of the notch then has a width of typically 1-10 kHz and is centered on the resonant frequency of an ion of interest. More generally, where the trapping RF frequency is f, then the auxiliary frequencies should be up to f/2.

against frequency.

Figures 7c and 7d are graphs showing variation of intensity against frequency for different pressures in the chamber of Figure 2b;

Figure 7e is a graph showing variation of resolution with pressure for different excitation voltages for the apparatus of Figure 2b;

Figure 8 is an isometric 3-dimensional view showing variation of the intensity of reserpine precursor ion with the auxiliary voltage;

Figures 9a and 9b are graphs showing similar plots to Figure 8 with the auxiliary voltage at the resonant frequency and 2-5 kHz below resonant frequency;

Figure 10 shows a variation of precursor and fragment intensity with excitation period; and

Figure 11 is a series of graphs demonstrating MS^3 in the apparatus of Figure 2b.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Referring first to Figure 1, a mass spectrometer is indicated generally by the reference 10. Ions are generated by an ion source 12, which is a pneumatically assisted electrospray, and pass through a dry nitrogen "curtain gas", indicated at 14. The ions then pass through an orifice in plate 16, and then through a further orifice in a skimmer 18, into a first quadrupole rod set Q0.

The rod set Q0 is located in a first chamber 22 which is connected to a turbo molecular pump, with the connection indicated at 24. Although not shown, in known manner, the turbo molecular pump 24 is backed up by a rotary vane pump, which can also be connected to the region between the orifice plate 16 and the skimmer plate 18. Alternatively the region between the orifice and skimmer plates 16, 18 can be evacuated by a separate rotary vane pump.

The turbo molecular pump 24 maintains a pressure of 7×10^{-3} torr (9.1×10^{-4} kPa) in the chamber 22, while a pressure of 2 torr (0.3 kPa) is maintained between

the orifice and skimmer plates 16, 18. The rod set Q0 has just an RF voltage applied to it, so that it operates as an ion guide.

Ions then pass through into a main chamber 26 of the mass spectrometer. Within the main chamber 26, there are located first, second and third quadrupole rod sets, indicated at Q1, Q2 and Q3. A detector 36 is provided at the exit from the final rod set at Q3.

As indicated at 30, a connection to a suitable turbo molecular pump would be provided, again backed by the same rotary vane pump that backs turbo molecular pump 24. The pump 30 maintains a pressure of 2×10^{-5} torr (2.6×10^{-6} kPa) in the main chamber 26.

The central quadrupole rod set Q2 is enclosed in a chamber or housing 28 and is provided with a connection for a gas (not shown), so that a higher pressure can be maintained typically at around 1-7 millitorr (1.3×10^{-4} to 9.1×10^{-4} kPa).

Now, in accordance with the present invention, the housing or enclosure 28 with the rod set Q2 forms a linear ion trap. For this purpose, conductive plates with apertures are provided at the ends of the housing 28, which may be either separate from the housing 28 or integral therewith. These comprise an entrance plate 32 and an exit plate 33. The plates 32, 33 are conductive, insulated from another and connected to voltage sources 34.

Downstream from the housing 28 is a third quadrupole rod set, Q3, configured as a mass analyzer. For operation as a conventional tripl quadrupole MS/MS system, the quadrupole rod sets Q0, Q1, Q2 and Q3 would be connected to conventional voltage sources, for supplying DC and RF voltages as required.

In use, ions generated from the ion source 12 pass into the quadrupole ion guide Q0. As noted, this is supplied with just RF voltages, to operate as an ion guide. Ions then pass through Q0 into the first quadrupole rod set Q1. This is supplied with suitable RF and DC voltages to operate as a mass filter, to select ions with a desired m/z ratio.

A mass selected precursor ion from the first rod set Q1 is then injected into the collision cell 28, to produce fragment ions as is

brevity, like components in Figure 2a are given the same reference numeral as in Figure 1, and description of these components is not repeated.

In Figure 2a, the time of flight device 40 is connected to the exit plate 33 of the collision cell 28. In known manner, the time of flight device 40 includes a connection 42 to a pump for maintaining a vacuum at 5×10^{-7} torr (6.5×10^{-8} kPa). It includes a repeller grid 44 and other grids indicated schematically at 46, for collecting ions entering the TOF 40 and transmitting a pulse of ions. The TOF device 40 here is a reflectron and includes grids 48 for reflecting the ion beam, which is then detected by a detector 49. A linear TOF may also be used, as shown in Figure 2b.

The apparatus in Figure 2a would be operated in an essentially similar manner to that of Figure 1. The principal difference is that the TOF can record 10^4 or more complete mass spectra in one second. Thus for applications where a complete mass spectrum of fragment ions is desired the duty cycle is greatly improved with a TOF mass analyzer 40 and spectra can be acquired more quickly. Alternatively, for a given measurement time, spectra can be acquired on a smaller amount of sample.

While three-dimensional (3-D) traps (IT) have been provided in spectrometers including a TOF final stage, a two-dimensional (2-D) trap has several advantages over the 3-D trap. Firstly, because there is no quadrupolar electric field in the z direction, the ion injection and extraction efficiencies can be nearly 100%. As fewer ions are lost in the processes of filling and emptying the trap the sensitivity of the Linear Ion Trap Time Of Flight Mass Spectrometer (LIT/TOFMS) can be greater than that of the IT/TOFMS (an ESI source, a 3-D ion trap mass spectrometer and a TOFMS).

Because of the increased trapping volume of the LIT, a greater number of ions ($N_{2\text{-}d}$) can be trapped in a LIT than in a 3-D trap ($N_{3\text{-}d}$). The increase in ion capacity is given by

$$\frac{N_{2\text{-}d}}{N_{3\text{-}d}} = \frac{r_0^2 l}{z_0^3} \qquad (1)$$

Reference will now be made to Figure 2b, which shows an alternative embodiment. This was designed without the initial, mass resolving quadrupole Q1, to provide experimental data on the performance of the LIT. It also includes a linear TOF section, to provide LIT/TOFMS.

The LIT/TOFMS was designed to be flexible with three modes of operation: (i) continuous flow-TOFMS, in which the products of ESI can be analyzed without trapping or fragmentation; (ii) trap-TOFMS, in which the combination of trapping and pulsing ions can be used to enhance instrumental duty cycle; and (iii) MS/MS-TOFMS in which the fragmentation spectra for isolated precursor ions are recorded via TOFMS. Switching between modes is a simple matter of changing the parameters which control timing, trap entrance and exit potentials, and excitation frequencies and amplitudes.

In Figure 2b, the spectrometer is indicated generally at 50. Ions are generated by pneumatically assisted electrospray at 52 and pass through a dry nitrogen curtain gas 54, a 0.25 mm diameter sampling orifice in an orifice plate 56, a 0.75 mm diameter orifice in the skimmer 58, and into a first RF-only quadrupole Q0. The region between the skimmer and the orifice is evacuated by a rotary vane pump as indicated at 62, to a pressure of 2 torr (0.3 kPa). A second quadrupole rod set is indicated at Q2. For consistency with Figure 2a, the designation Q2 is used, although there is no Q1 in Figure 2b.

The RF-only quadrupoles Q0, Q2 are separated by a 1mm diameter interquad aperture 64 (IQ). The first quadrupole, Q0, is 5 cm long and the second Q2, which acts as the LIT, is 20 cm long. Both quadrupoles have field radii of 4.0 mm and are operated by the same main RF drive, which has a maximum V_{rf} of 5000 V and a drive frequency, $\Omega = 2\pi f$ where f is 1 MHz. There are 12.5 pF capacitors between the RF drive supply and the rods of Q0; thus the RF voltage on Q0 is ca. one half that of Q2. The DC offsets of the quadrupoles are individually set and for all the experiments here these are Q0=10 V and Q2=0 V. The pressure in the LIT can be varied from 1.5 to 7.0 mTorr (2×10^{-4} to 9.1×10^{-4} kPa) by adding gas. The region surrounding the LIT

provided by Q2 is connected to a turbomolecular pump, as indicated at 66. The LIT chamber is indicated at 68.

A TOF chamber 70 is coupled orthogonally to the LIT chamber 68 via four lenses, L1-L4. L1 (aperture diameter 0.75 mm) serves as the exit of the LIT chamber 68 and the differential pumping aperture between the LIT and the TOF chambers. The three lenses, L2, L3 and L4, have apertures of 2 mm diameter and are used to focus the ion beam into the source region of a two stage, 1 m long, TOFMS. The TOF chamber 70 is held at a pressure of 1.2 x 10^{-6} torr ($1.6x10^{-7}$ kPa) or less by a turbomolecular pump. Separate rotary vane pumps are used to pump the region between the orifice and skimmer and to back the turbo pumps.

In the TOF source region, in known manner there are a repeller grid 72, a middle TOF grid 74 and a final TOF grid 76. The ion source was operated near ground potential and the flight tube was floated at a negative high potential, typically 2.0kV. To reduce distorting effects of the floating voltage a shielding grid 78 was placed 4.2 mm behind the middle TOF grid 74. An additional shielding grid 80 was placed around the repeller grid 72 and the middle TOF grid to reduce the effects of stray fields on ions entering the source region. Ions are accelerated in the TOF in a direction orthogonal to that of the quadrupoles. Thus, the system is termed an orthogonal acceleration TOF (oa-TOF).

There is no potential difference between the Q2 offset and the source region, thus the axial ion energy in the TOF source is < 1 eV. The repeller grid 72 of the TOF is pulsed from an offset of 0 V to an amplitude of ≈ 200-300 V using a high voltage (HV) pulser (rise time < 18 ns). The amplitude of the HV pulse is adjusted to achieve maximum resolution for the ion acceleration energy. Because the ions enter the source region midway between the repeller grid 72 and grid 74, the acceleration energy is given by one half of the amplitude of the HV pulse minus the negative float potential. The experimental HV pulse amplitudes that gave the best resolution were found to equal those calculated to give space focussing for the set acceleration energies. The HV pulse width is set to be greater than

substantial loss in resolution in the TOFMS. Nitrogen was added to the flight tube to increase the pressure over the range 1.2×10^{-6} torr (1.6×10^{-7} kPa) to 5×10^{-5} torr (6.5×10^{-6} kPa), corresponding to a decrease in the mean free path for the +13 charge state of cytochrome *c* (collision cross section ~ 1700 Å^2) from ~ 106 cm to ~ 4 cm. The resolution in the TOFMS spectrum degraded from 600 to 30 with a ca. 25 x increase in the number of collisions. At 1.2×10^{-6} torr (1.6×10^{-7} kPa), the probability of the ion undergoing zero collisions in the flight path is 52% and of one collision 34%. Extrapolation of a graph of resolution vs. λ^{-1} shows that at zero pressure the resolution is improved ca. 6% over that of 1.2×10^{-6} torr (1.6×10^{-7} kPa).

A schematic of the RF operation for the LIT is shown in Figure 3. The master clock for the LIT/TOFMS is provided by a two channel arbitrary waveform generator 82 (AWG). Each channel of the AWG 82 provides a maximum amplitude (0 to peak) of 12 V. The AWG 82 is connected to an auxiliary drive (Aux. Drive) 84, which in turn is connected by a bipolar transformer 85 to the y rods. A main RF drive 86, as shown, is connected directly to the x rods, with one connection being through the transformer 85 to the y rods.

As shown in Figure 4 the complete MS/MS cycle takes 20 ms to complete. It involves changing the potentials on the interquad aperture (IQ) 64 and exit aperture L1, control of the auxiliary driver 84 which connects the output of the AWG 82 to the quadrupole rods Q2, and the TOFMS pulsing (TOF).

The first phase of the cycle is ion injection. A synchronization pulse from the AWG 82 triggers a pulse generator (not shown) which controls the potential on IQ 64, which is maintained at a potential (-7 V) indicated at 100 to pass ions for a set injection time (typically 5 ms as shown in Figure 4) and a stopping potential 102 (12 V) for the remaining 15 ms of the scan. In addition this injection time serves as a thermalization period. For the ions that were studied, fragmentation spectra were independent of orifice skimmer potential difference, suggesting that any ion heating in the ion sampling region has equilibrated during the

7b with q_y=0.5, only ions with $\frac{m}{z}$ >345 will have a Mathieu parameter for motion in the y direction, q_y, of <0.9 and thus stable trajectories in the trap. Lower mass fragments will have unstable trajectories and thus, even if formed by the resonance excitation process, cannot be detected in the TOFMS. This decreases the fragmentation efficiency, but this is an inherent feature of CID in any ion trap.

Figure 7c and 7d compare resonant excitation curves, which show precursor and fragment ion intensities for renin substrate as a function of the frequency, ω_a of the auxiliary voltage for ω_a near ω_o (the fundamental resonant frequency of the system) for pressures of (a) 7 mTorr (9.1×10^{-4} kPa) and (b) 1.5 mTorr (2×10^{-4} kPa) respectively in the chamber 68. The data of Figure 7c is the same as Figure 7a, and references 120c, 120d, 124c, 124d are used to identify the curves in these Figure 7c, 7d. In each case a broadband waveform constructed to isolate the precursor ion, (the +3 charge state of renin substrate $\frac{m}{z}$=587), was applied for 4 ms prior to the irradiation with the auxiliary voltage. Figure 7c again shows, for a pressure of 7 mTorr (9.1×10^{-4} kPa) the intensity of the precursor ion 120c falling to a minimum, as the intensity of the sum of the fragments 124c reaches a maximum. Corresponding curves 120d, 124d are shown in Figure 7d, for the precursor ions and the fragment ions for operation at a pressure of 1.5 mTorr (2×10^{-4} kPa). The achieved resolution at 7 mTorr (9.1×10^{-4} kPa) was ~70 and at 1.5 mTorr (2×10^{-4} kPa) was approximately ~230. The major difference in the excitation parameters for the two pressures is the amplitude of the auxiliary voltage. At 7 mTorr (9.1×10^{-4} kPa) a 0-peak voltage of 1500 mV was required to achieve fragmentation and ejection while at 1.5 mTorr (2×10^{-4} kPa) the same phenomena were observed with 300 mV.

Figure 7e demonstrates the achieved resolutions for different excitation voltages over a range of pressures. Resolution remains essentially constant as a function of pressure at each amplitude. Clearly the use of a lower auxiliary voltage amplitude is the dominant factor in the observed improved resolution at the lower pressure.

ion ejection and ion fragmentation. For ejection the important parameters are q_y, the mass and charge of the precursor ions, the pressure and mass of the collision gas, and the collision cross section of the precursor ion, as well as the duration and amplitude of the auxiliary voltage. Fragmentation, however, is also dependent upon the structure of the precursor ion, the effectiveness of the transfer of kinetic energy to internal energy through collisions, and the time scale for the unimolecular dissociation of the precursor ion. The details of this competition have been discussed for 3-D ITMS previously. There follows a discussion on tests to demonstrate how operating parameters for the apparatus of Figure 2b affects competition between ejection and fragmentation.

Figure 8 shows the effect on singly charged reserpine ions of increasing the amplitude of the auxiliary voltage, as plotted against intensity and channel number. While a threshold voltage is necessary to induce fragmentation, as the amplitude increases ejection dominates and no fragmentation is observed. The precursor ion is indicated at 130, and fragments at 132, 134. Figure 9a shows a similar plot for the same experiment for the +3 charge state of renin substrate, with the precursor indicated at 136 and the sum of the fragments at 138. Figure 9b demonstrates the effect of increasing the amplitude of the excitation voltage at $\omega_a=\omega_o$-2.5 kHz, with the precursor indicated at 140 and the sum of the fragments at 142. The extent of fragmentation increases by 50% and the excitation amplitude for which fragmentation is one half of its maximum value increases by 40% when off resonant excitation is used. Similar increases in the extent of fragmentation and the amplitude for which fragment ions could be detected were observed for all ions and charge states examined.

The fragmentation spectrum recorded with low amplitude resonant excitation (A a=1.5 V, $\omega_a=\omega_o$) was similar to that recorded in a triple quadrupole mass spectrometer system with 15 eV laboratory injection energy and a 20 cm long collision cell filled with 1 mTorr (1.3×10^{-4} kPa) of Krypton (The triple quadrupole is described in Y-L. Chen, B.A. Collings, and D.J. Douglas,

with other ions being ejected. Finally, the isolated peak is fragmented through the application of a low amplitude sinusoidal oscillation for another 1 ms. The fragments of the isolated fragment at $\frac{m}{z}=726$ are shown in Figure 11e. The total trapping time for the MS^3 process was 10 ms, giving a cycle time for MS^3 of 22 ms, with 70 TOFMS scans in each MS^3 cycle. As is shown the spectral intensity is lower by a factor of 100 in the MS^3 process.

Nitrogen was used as the collision gas because it flowed into the quadrupole from the curtain gas region. A pressure of 7 mTorr (9.1×10^{-4} kPa) was initially used because this previously was found to give optimum collisional focussing for a single pass through an RF quadrupole of similar length. These choices however, somewhat limited the performance of the LIT. There are experimental tradeoffs when using a heavier neutral as a collision gas. The heavier neutral results in a larger center of mass collision energy for a given ion energy, and thus both a greater energy exchange in collisions and more scattering. While the larger collision energy exchange results in fragmentation occurring on a shorter time scale, the increase in the extent of scattering can degrade excitation resolution. The inelastic collisions between the gas and the precursor ion act as a "frictional force" which dampens the forced oscillation of a harmonic system and the width of the power absorption is related to the dampening of the ion motion. Lowering the pressure and mass of the gas is expected to lower the frictional force, thus narrowing the width of the power absorption and thereby increasing the possible excitation resolution. This applies to both the broadband excitation waveform and the resonant excitation resolution.

CLAIMS:

1. A method of analyzing a stream of ions, the method comprising:

(1) subjecting a stream of ions to a first mass analysis step, to select ions having a mass-to-charge ratio in a first desired range;

(2) passing ions in the selected range into a radio frequency linear ion trap (Q2) containing a gas;

(3) trapping the selected ions in the linear ion trap (Q2) and exciting the ions to cause collisions with the gas and fragmentation;

(4) subjecting the fragmented ions to a secondary excitation, different from the first excitation, to cause excitation and fragmentation of selected fragment ions; and

(5) passing the ions out of the linear ion trap (Q2) and subjecting the ions to a further mass analysis step to determine the mass spectrum of the ions.

2. A method as claimed in Claim 1, which includes, prior to the additional step of secondary excitation, applying a signal to the linear ion trap (Q2), to isolate ions having a mass-to-charge ratio in a second desired range, wherein the secondary excitation step comprises exciting ions in the second desired range.

3. A method as claimed in Claim 2, which includes, while trapping the ions in the linear ion trap (Q2), effecting multiple cycles of:

(1) isolating ions having a mass-to-charge ratio in a further desired range; and

(2) exciting the isolated ions in the further desired range to cause fragmentation.

4. A method as claimed in Claim 1, 2, or 3 wherein step (2) comprises passing the ions into the linear ion trap (Q2) with sufficient energy to promote collision induced dissociation, the said energy providing the

excitation of step (3), whereby step (3) comprises applying a signal to the linear ion trap (Q2) to trap ions before subjecting the ions to the further mass analysis of step 4).

5. A method as claimed in Claim 1, 2, 3 or 4 which comprises exciting the ions in the linear ion trap (Q2) by providing an additional signal to the linear ion trap (Q2) to effect resonant radial excitation of ions.

6. A method as claimed in any preceding claim, wherein the further mass analysis step of step (5) is carried out in a quadrupole mass analyzer (Q3).

7. A method as claimed in any of claims 1 to 6, wherein the further mass analysis step of step (5) is carried out in a time of flight mass analyzer (40).

8. A method as claimed in Claim 7 wherein the further mass analysis step is carried out in a time of flight mass analyzer (40) arranged with its axis perpendicular to the axis of the linear ion trap (Q2).

9. A method as claimed in Claim 1 wherein each mass analysis step is carried out in one of: a linear quadrupole (Q3); a linear time of flight analyzer (40); a reflectron time of flight analyzer; a single magnetic sector analyzer; a double focusing two sector mass analyzer having an electric sector and a magnetic sector; a Paul trap; a Wien filter; a Mattauch-Herzog spectrograph; ion cyclotron mass spectrometer; and a Thomson parabolic mass spectrometer.

10. A method as claimed in Claim 6, 7, 8, or 9, wherein the first mass analysis step is carried out in a quadrupole mass analyzer (Q1) which is coaxial with the linear ion trap (Q2).

11. A method as claimed in Claim 1, which includes, prior to exciting the ions in step (3), subjecting the trapped ions to a signal comprising a

plurality of excitation signals uniformly spaced in the frequency domain and having a notch, wherein the notch covers a desired frequency band and there are no excitation signals in the frequency band of the notch, and wherein the excitation signals have sufficient magnitude to excite and eject ions except for ions having an excitation frequency within the frequency band of the notch.

12. A method as claimed in Claim 11, which comprises applying a combination of signals comprising sine waves and with frequencies up to f/2, where f is the frequency of the trapping RF.

13. A method as claimed in Claim 11, which comprises applying a combination of signals having sine waves with frequencies in the range 10 to 500 kHz and spaced at 500 Hz intervals, and the frequency band of the notch has a width of 1-10 kHz and is centered on the resonant frequency of an ion of interest.

14. A method as claimed in Claim 11, 12 or 13 which includes, after selection of a desired ion, exciting the desired ion with a signal comprising a sine wave at or near the resonant frequency of the ion.

15. A method as claimed in Claim 7, which includes providing an exit lens (33) between the linear ion trap (Q2) and the time of flight device (40), and lowering the voltage on the exit lens (33) to permit ions to pass into the time of flight device (40), the method further comprising providing a signal to a repeller grid (44) of the time of flight device (40), to cause the time of flight device (40) to scan at a desired rate.

16. A method as claimed in Claim 15, which comprises passing ions, in step (2), into the linear ion trap (Q2) for a period of substantially 5ms subjecting the ions in the linear ion trap (Q2) to an excitation signal to excite and eject undesired ions for a period of substantially 4ms, exciting the desired ions for a period of substantially 4ms and passing the ions out of the linear ion trap (Q2) and scanning the time of flight device (40) for substantially 7ms.

17. An apparatus (10), for effecting mass analysis and fragmentation of an ion stream, the apparatus comprising:

an input (12) for an ion stream;

a first mass analyzer (Q1);

a radio frequency linear ion trap (Q2);

a final mass analyzer (Q3); and,

an auxiliary drive (84) connected to the radio frequency linear ion trap (Q2) for effecting multiple excitation steps.

18. An apparatus as claimed in Claim 17, wherein the first mass analyzer (Q1) comprises a quadrupole mass analyzer.

19. An apparatus as claimed in Claim 17 or 18, wherein the final mass analyzer (Q3) comprises a quadrupole mass analyzer, and the first mass analyzer (Q1), the linear ion trap (Q2) and the final mass analyzer (Q3) are axially aligned with one another.

20. An apparatus as claimed in Claim 17 or 18, wherein the final mass analyzer (Q3) comprises a time of flight device (40).

21. An apparatus as claimed in Claim 19 or 20, wherein the linear ion trap (Q2) includes a multipole rod set.

22. An apparatus as claimed in Claim 21, wherein the linear ion trap (Q2) comprises a quadrupole rod set and wherein the rods of the mass analyzers (Q1 and Q3) and of the linear ion trap (Q2) have substantially similar radii and substantially similar spacings.

23. An apparatus as claimed in Claim 17, wherein each of the first analyzer (Q1) and the final analyzer (Q3) comprise one of: a linear quadrupole; a linear time of flight analyzer (40); a reflectron time of flight analyzer; a single magnetic sector analyzer; a double focusing two sector mass analyzer having an electric sector and a magnetic sector; a Paul trap; a Wien filter; a Mattauch-Herzog spectrograph; an ion cyclotron mass spectrometer; and a Thomson

parabolic mass spectrometer.

24. An apparatus as claimed in Claim 23, wherein the linear ion trap (Q2) includes a multipole rod set.

25. An apparatus as claimed in Claim 17 or 22, wherein the linear ion trap (Q2) has a pair of opposed x rods and a pair of opposed y rods, wherein a main RF drive (86) is connected to the x and y rods (88 and 90) of the linear ion trap (Q2), and wherein the auxiliary drive (84) is connected to at least one pair of rods of the linear ion trap (Q2).

26. An apparatus as claimed in Claim 25, wherein the auxiliary drive (84) is connected to the y rods (90) of the linear ion trap (Q2) through a transformer (85), and wherein the main RF drive (86) is connected directly to the x rods (88) of the linear ion trap (Q2) and, through a coil of the transformer (85) to the y rods (90).

27. An apparatus as claimed in Claim 25, which includes an arbitrary waveform generator (82) connected to the auxiliary drive (84), for applying a selected waveform to the linear ion trap (Q2) to excite ions therein.